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Molecular Characterization of a Carbapenem-Hydrolyzing Class A β-Lactamase, SFC-1, from *Serratia fonticola* UTAD54

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An environmental isolate of *Serratia fonticola* resistant to carbapenems contains a gene encoding a class A β-lactamase with carbapenemase activity. The enzyme was designated SFC-1. The bla_{SFC-1} gene is contained in the chromosome of *S. fonticola* UTAD54 and is absent from other *S. fonticola* strains.

The prokaryotic species *Serratia fonticola*, a species of the family *Enterobacteriaceae*, includes organisms that occur naturally in environmental waters (3); occasionally, some strains cause infections in humans (15). A recent study on the natural antimicrobial susceptibilities of strains of *Serratia* species (23) showed that *S. fonticola* expresses both a chromosomally encoded extended-spectrum class A β-lactamase and a species-specific AmpC β-lactamase. The class A enzyme corresponds to the previously characterized β-lactamase SFO-1 (13), and the homologous sequence FON-A (GenBank accession no. AJ251239) is common to *S. fonticola* (19).

In a previous report (18), an environmental isolate designated *S. fonticola* UTAD54 was shown to be resistant to carbapenems. This phenotype could be attributed to a gene encoding a class B metallo-enzyme (Sfh-I) that was isolated from a genomic library (18). An additional screening of the library was done on Luria-Bertani plates supplemented with ampicillin (50 μg/ml) and kanamycin (30 μg/ml) to select for inserts and the vector, respectively. Some of the clones obtained were negative when screened by PCR using primers (18) for genes homologous to SFO-1. A recombinant plasmid containing a 1.8-kb insert was selected for study and designated pIH18.

Characterization of a new β-lactamase gene. Plasmid DNA was prepared with a Qiaprep kit (Qiagen, Courtaboeuf, France), and both strands of the insert were sequenced on an ABI cycle sequencer A373 (Applied Biosystems/Perkin-Elmer, Foster City, Calif.) using the ABI Prism dye terminator kit.

Analysis of sequence data revealed the presence of an open reading frame of 927 bp encoding a 33.6-kDa protein containing 309 amino acids (Fig. 1). Four nucleotides upstream of the ATG codon have the sequence AAGG, a putative ribosome-binding site (RBS). A typical −10 region (TATACT) was identified upstream from the RBS; no conserved −35 region could be assigned. Downstream, the open reading frame is a palindromic sequence (Fig. 1) which might form a hairpin loop in the mRNA, typical of a transcription terminator. The overall G+C content of bla_{SFC-1} (45.3%) is characteristic of genes of *Enterobacteriaceae*.

A similarity search was performed with BLAST (1). SFC-1 had the highest similarity to the class A carbapenemases, in particular KPC-1 (62% identical) from *Klebsiella pneumoniae* (21), Sme-1 (58%), NMC-A (59%), and IMI-1 (59%) (12). Lower similarity scores were returned for the other class A β-lactamases. No putative LysR-type regulator gene was identified upstream of the bla_{SFC-1} gene, whereas such regulators are transcribed upstream of the genes coding for NMC-A, Sme-1, and IMI-1 (7, 8).

The software SignalP (11) identified a bacterial signal peptide of 26 amino acids in the amino-terminal sequence (Fig. 1). Cleavage of this signal peptide would yield a mature protein of 30.7 kDa with a pI of 7.95.

Within the mature protein, a serine-serine-phenylalanine-lysine tetrad (S-S-F-K) was found, as was a lysine-threonine-glycine (KTG) motif. These motifs (SXXK and KTG) are characteristic of serine β-lactamases (16, 22). The nine invariant residues typical of class A enzymes (G45, S70, K73, P107, S130, D131, A134, E166, and G236) are conserved in the SFC-1 sequence. From the residues suggested to be important for class A carbapenemase activity (C69, S70, K73, H105, S130, R164, E166, N170, D179, R220, K234, S237, and C238), only S237 was not conserved in the SFC-1 sequence.

The deduced amino acid sequence of SFC-1 was aligned to the sequences of 15 class A β-lactamases, using CLUSTAL W at the European Molecular Biology Laboratory website (http://www.embl-heidelberg.de/). The enzymes and their GenBank accession numbers were the following: KPC-1 (24) from *K. pneumoniae* (AAG13410), IMI-1 (17) from *Enterobacter cloacae* (AAR93461), Sme-1 (9) from *Serratia marcescens* (CAA82281), OXY-1 (2) from *Klebsiella oxytoca* (P22391), CITDI (14) from *Citrobacter diversus* (S19006), YENT (20) from *Yersinia enterocolitica* (Q01166), CTX-M-12 (19) from *K. pneumoniae* (AAG34108), CTX-M-14 (19) from *Escherichia coli* (CAC95170), Toho-1 (6) from *E. coli* (BAA07082), SFO-1 (6) from *E. cloacae* (BAA76882), FONA-3 from *S. fonticola* (CAB61639), SER_FON (13) from *S. fonticola* (P80545), TEM-1 (24) from *E. coli* (AAR25033), SHV-1 (24) from *E. coli* (P14557), and CARB-3 (4) from *Pseudomonas aeruginosa* (P37322). The dendrogram shown in Fig. 2 was derived from

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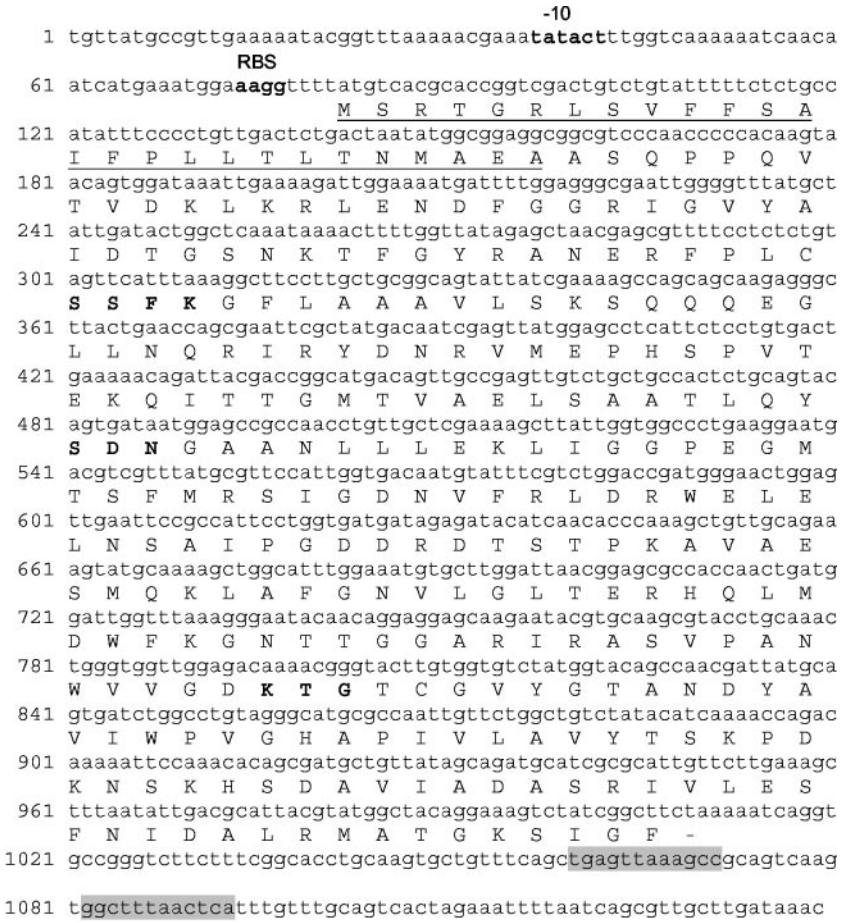

FIG. 1. Nucleotide and deduced amino acid sequences of the SFC-1 gene and its upstream and downstream regions. The putative −10 region and a potential RBS are in bold. The inverted repeat sequences that can act as a terminator of transcription are shaded. The putative signal peptide for protein secretion is underlined. Amino acids that correspond to conserved domains of class A β-lactamases are shown in bold.

the alignment: SFC-1 clusters to the class A carbapenemases and is more closely related to a subgroup that includes the enterobacterial enzymes of extended hydrolytic spectrum.

Susceptibility to antibiotics. The MICs were determined by the E-test method (Biodisk, Solna, Sweden), and susceptibility categories were allocated according to those described in reference 10. Table 1 shows the MICs for *S. fonticola* UTAD54, *E. coli* transformed with plasmid pIH18, and untransformed *E. coli*. The DNA insert encoding SFC-1 when replicating in *E. coli* confers resistance to ampicillin, amoxicillin, piperacillin, cephalothin, and aztreonam and reduced susceptibility to meropenem and imipenem, and its activity is inhibited by the class A β-lactamase inhibitors. Such a resistance pattern is characteristic of a carbapenem-hydrolyzing class A β-lactamase.

Chromosomal location of class A β-lactamases in *S. fonticola* UTAD54. DNA from *S. fonticola* UTAD54 embedded in agarose was digested with I-CeuI (New England Biolabs, Hertfordshire, United Kingdom), and the resulting fragments were separated on a CHEF-DRII apparatus (Bio-Rad, Richmond, Calif.) (5). Six fragments were generated. After immobilization on nylon membranes, the I-CeuI-generated fragments were hybridized with three different probes: an rRNA gene probe, a probe specific to the naturally occurring class A β-lactamase (SFO-1), and a bla_{SFC-1} probe.

The probes were generated by PCR amplification in the presence of digoxigenin (Roche Molecular Biochemicals, Indianapolis, Ind.). For rRNA genes and the SFO-1 gene, the primers were previously reported (18). Specific primers were designed to amplify the bla_{SFC-1} gene, SfcF (5′-GATCTCGAGA

TABLE 1. MICs of antibiotics for *S. fonticola* UTAD54, *E. coli* XL2 Blue(pIH18), and *E. coli* XL2 Blue (reference strain)

Antibiotic[a]	MIC (μg/ml)		
	S. fonticola UTAD54	*E. coli* XL2 Blue(pIH18)	*E. coli* XL2 Blue
Ampicillin	>256	>256	2
Amoxicillin	>256	>256	4
Amoxicillin-CLA	12	32	3
Piperacillin	32	64	0.75
Piperacillin-TZB	0.38	6	0.5
Cephalothin	>256	>256	6
Cefepime	0.032	0.75	0.032
Cefotaxime	2	1	0.064
Ceftazidime	0.064	1	0.19
Aztreonam	1.5	64	0.19
Meropenem	>32	0.38	0.008
Imipenem	>32	4	0.125

[a] CLA, clavulanic acid; TZB, tazobactam.

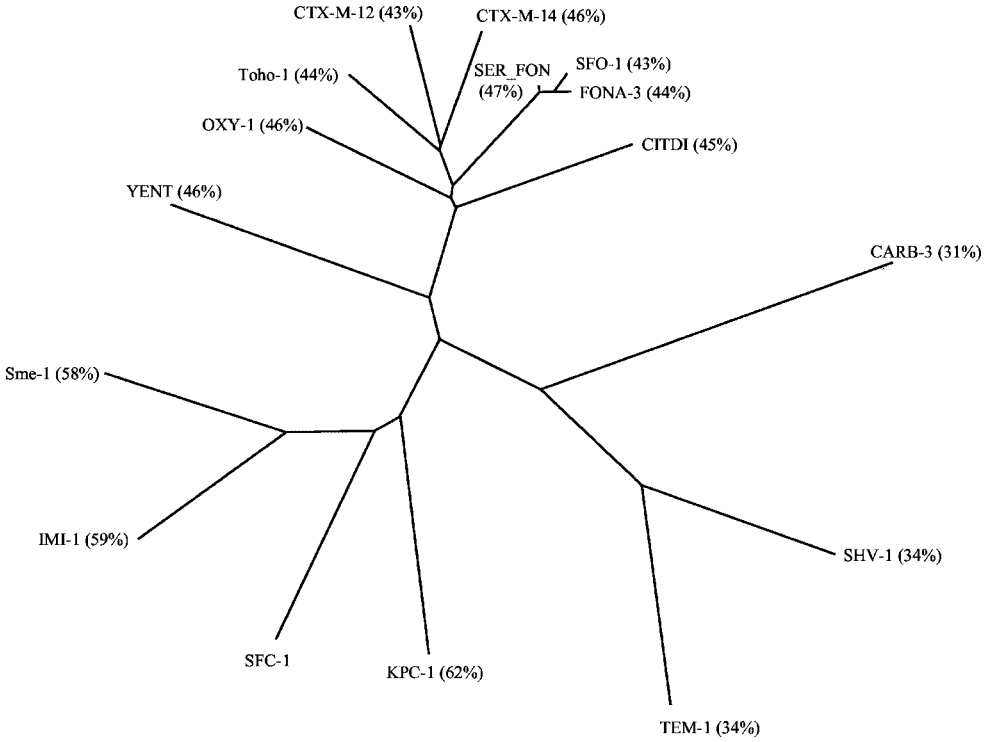

FIG. 2. Dendrogram obtained from the multiple sequence alignment of 15 class A β-lactamases. The percent identity between the amino acid sequence of each enzyme and that of SFC-1 is indicated in brackets.

ATGTCACGCACCGGTCGACTG-3′), and SfcR (5′-GATGA ATTCTTAGAAGCCGATAGACTTTCC-3′). The probes for the SFC-1 and SFO-1 genes revealed two different I-CeuI bands, as shown in lanes 1 and 2 of Fig. 3; the probe for rRNA genes hybridized to the six I-CeuI bands (lane 3). These results thus indicate that both β-lactamase genes are chromosomally encoded and apart from each other. Hybridization of the probe for bla_{SFC-I} with DNA from *S. fonticola* strains LMG 7882^T, DSM 9663, and CIP 103850 did not detect homologous sequences in these genomes.

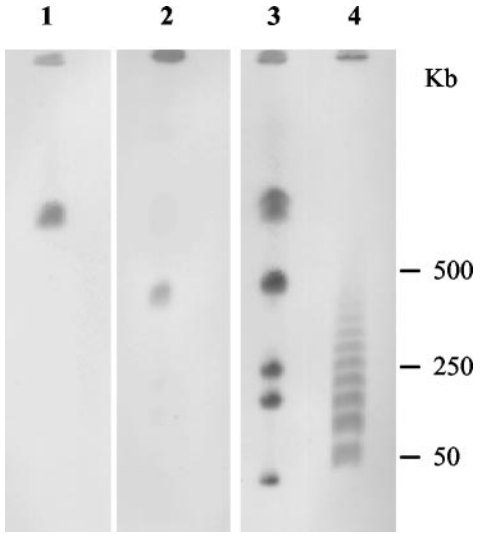

FIG. 3. Hybridizations to I-CeuI fragments generated from the genome of *S. fonticola* UTAD54 and separated by pulsed-field gel electrophoresis. Lane 1, hybridization with SFC-1 probe; lane 2, hybridization with probe for naturally occurring class A β-lactamases of *S. fonticola*; lane 3, hybridization using a probe for rRNA genes; lane 4, concatemers of phage lambda DNA.

Concluding remarks. *S. fonticola* UTAD54 is an exceptional strain, carrying the naturally occurring β-lactamases of *S. fonticola* and different classes of carbapenemases, SFC-1 and the previously reported metallo-enzyme Sfh-I. Those enzymes are not present in other *S. fonticola* strains. These exceptional characteristics could be the result of the acquisition of a genetic element by horizontal gene transfer.

Nucleotide sequence accession number. The nucleotide sequence reported here was deposited in GenBank under accession number AY354402.

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